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EFFECT OF SALT STRESS ON ROOT PLASTICITY AND EXPRESSION OF ION TRANSPORTER GENES IN TOMATO PLANTS

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ABSTRACT

One of the most adverse abiotic stresses affecting agriculture worldwide is soil salinity. Salt stress results in decreasing plants growth, yield and, consequently, affecting the domestic and global economy. Tomato is an economically important salt-sensitive vegetable grown all over the world, and is considered the main vegetable grown in Egypt in terms of area and production. The first part of this study aims to evaluate the plasticity of Root System Architecture (RSA) of Egyptian tomato cv. Agyad-16 under salt stress. For this purpose, minirhizotron system was used to monitor the growth and RSA of the plants using soil substrate, where tomato seedlings were irrigated with either tap water (control) or 125 mM NaCl (salt treatment). Salinity significantly decreased main root length (MRL), lateral root length (LRL), number of lateral roots and total root size. In addition, there was a significant inhibition in root growth rate and fresh weight of roots. In the second part of this study, the aim was to examine the response of potted cv. Agyad-16 plants to salt stress at an early growth stage. In potted plants subjected to control (tap water) or salt stress (125 mM NaCl solution) treatments, salt stress increased Na+ contents and Na+/K+ in both leaves and roots, while the content of K+ slightly decreased. Also, a significant increase in proline accumulation was detected in response to salt treatment, while total chlorophyll content were only slightly affected. At the molecular level, salt treatment resulted in a slight increase in the gene expression of both SlSOS1 and SlNHX3 cation transporters in roots, both triggered as a tolerance mechanism against the high salt levels in the external environment. All in all, our results are in harmony with similar previous studies on other tomato accessions, indicating that Egyptian tomato cv. Agyad-16 seems to be mildly sensitive to salt stress.

KEYWORDS: Salinity, Salt Stress, Ion Transporter Genes, Tomatoes

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Original Article

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world. In many countries its fruits are an important source of vitamins, minerals and antioxidants compounds in human food. Tomato is a moderately salt tolerant crop and is widely cultivated even in areas with salt affected soils or irrigation water. In Egypt, fruit and vegetable crops occupy a key point in agriculture and their importance is growing steadily, where vegetable consumption is one of the highest in the world at more than 185 kg/year. Egypt ranks 5th amongst the highest tomato producing countries in the world and the total production in Egypt were estimated to be 9,204,097 tons in 2010 (FAOSTAT 2010).

In addition, not only is tomato the main vegetable crop in Egypt in terms of production, but also tomato-growing area is about 32% of the total vegetable growing area, approximately estimated to be 200,000 ha. Its production represents 35 % of total vegetable production (FAOSTAT 2010).

During their life cycle, plants face different biotic and abiotic stresses. Among abiotic stresses, salinity is one of the most widely spread leading to big losses in plant yield and profits. One fifth of the irrigated land worldwide is affected by salinity and this percentage is expected to increase in near future. Due to the increased population all over the world, food production must increase to fill the gap between production and consumption. Additional challenges facing food production include progressive salinization of irrigated lands which affect negatively crop plants growth, where most of crops are considered salt-sensitive. Plant growth is affected negatively or inhibited to death when plants are exposed to low concentrations of NaCl (40 mM) in sensitive plants like Arabidopsis and rice. Salinity affects plants by different ways at the cellular level; it imposes osmotic stress, ion toxicity and cause nutrient imbalance in the plant. At the whole plant level, high amounts of salt in the soil negatively affects plant germination, growth, development and yield (Munns and Tester 2008; Manaa *et al*. 2011).

Nevertheless, plants possess tolerance strategies that enable them to cope with these adverse factors triggered by salinity. When plants are exposed to salinity stress, many changes in their metabolic pathways occur leading plants to acquire tolerance to salt stress. One of these metabolic changes is the increase in compatible solutes which are synthesized in the cell to protect plants in response to salinity. For example, salinity stress causes the accumulation of sugars, amino acids, non-toxic compounds and proline in plants including tomato (Fujita et al. 1998). These compounds play an important role in cell osmoprotection by maintaining turgor and osmotic adjustment (Conde *et al.* 2011).

Another strategy that plants use to cope with salinity stress is to limit the presence of Na^+ ion in the cytosol, which causes the main damage to plant cell. Plant cells, thus, have the ability to either minimize the amount of Na+ entering the plant, via the roots (Munns and Tester 2008),or sequester them within the vacuole where Na+ causes less harm (Parida and Das 2005). Several genes have been shown to play a role in the control of Na+ movement throughout the plant. For example, HKT (Ali *et al*. 2012), SOS (Shi *et al*. 2002), NHX (Gaxiola *et al*. 1999), AVP (Gaxiola *et al*.2001) and AHA (Apse *et al*. 1999) gene families encompass such genes. The plasma membrane Na^+/H^+ antiporter AtSOS1 shows its expression in the epidermal cells of the root tissue (Shabala and Cuin 2008), where it excludes Na^+ into the external medium. Also, SOS1 transporter is implicated in reducing the quantity of Na^+ translocated to the shoots *via* the transpiration stream (Shi *et al*. 2000; Qiu *et al*. 2002b; Shi *et al*. 2002). Another example of cation transporters is the vacuolar Na^+ and K^+/H^+ antiporter AtNHX1 (Venema *et al*. 2003). This transporter is very important in salinity tolerance and can detoxify the cytoplasm by sequestering Na^+ into the vacuole (Gaxiola *et al.* 1999).

Roots play important roles in plants life. They are responsible of up-taking water and nutrients from soil and mechanically anchoring and supporting plants. Roots are the first plant organ that faces any adverse conditions occurring in soil like drought, flooding and salt stress. Their response to stresses start with sensing the adverse factor then start signal transduction cascade leading to proper response towards the stress. Roots, thus, have the sufficient plasticity to modulate their growth in response to the adversely surrounding conditions. This alternation in their architecture enables them to grow properly in a large spectrum of unfavorable conditions. Unfortunately, Root System Architecture (RSA) did not receive a lot of attention from plant breeders as an important criterion for selection in crop development programs to potentially increase yield due to its difficulty and high cost of monitoring. Understanding how roots can alter their RSA for

optimum growth will help to exploit and manipulate root characteristics to both increase plant yield and tolerate adverse conditions. In reference to tomatoes' case, the root system consists of a main root (MR) and several lateral roots (LR). Salt stress affects the main and lateral roots in different ways based on salt concentration. Lower concentrations of salt were found to slightly induce MR and LR, while on the other hand, higher concentration of salt resulted in reduction of both MR and LR (Wang *et al* 2009).

The present study was conducted to examine the effect of salinity on RSA and physiology of Egyptian cultivar Agyad-16, one of the most cultivated and promising Egyptian hybrids. In addition we studied the expression level of *SOS1* and *NHX3* genes in roots of tomato plants under salt stress to asses at a molecular level its degree of salinity tolerance.

MATERIALS AND METHODS

Plant Material

The present study was carried out throughout March-May 2015 in the Plant Physiology Division, Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Egypt. Egyptian tomato hybrid cv. Agyad-16 was obtained from Agricultural Research Center, Giza, Egypt. The seeds of tomato were washed three times with tap water and left to sterilize in commercial sodium hypochlorite solution of 1% for 10 min and then rinsed three times with distilled water. Seeds were finally left to germinate for five days in Petri dishes lined with water-soaked cotton fiber.

Experimental design

A minirhizotron culturing system was used to allow a non-destructive study of shoot and root development during a certain period of vegetative growth. The minirhizotron used in this experiment consisted of two glass sheets of 30 x 30 cm separated by a 3 mm wide glass separator. The inner space available for culture substrate was approximately 250 cm^3. The thin layer of substrate used in the minirhizotron directed the roots to grow in 2D conditions and, thus, facilitated the monitoring and measurement of root system morphology parameters and RSA. The substrate of minirhizotron consisted of sieved peat moss and fine sand mixture (2:1). Two germinated tomato seeds were planted at approximately equal spacing from each other per minirhizotron. Five minirhizotron plate replicates were used per treatment. The minirhizotron plates containing the seedlings were placed vertically under the angle of 70° in the growth chamber and covered with aluminum foil to provide dark conditions for roots. All minirhizotron plates were kept in a growth chamber under long day conditions (25 °C, 70% humidity, 16/8h light/dark cycle).

The pot experiment was done to repeat the minirhizotron experiment in conditions similar to the field. Also, large root and shoot biomass were needed to analyze contents of sodium and potassium, photosynthetic pigments, proline, and most importantly for RNA extraction for qRT-PCR of *SOS1* and *NHX3* genes analyzed in this work. The pot experiment was placed in the greenhouse of the Department of Agricultural Botany during the month of April 2015. Three 5 L volume pots were used per treatment for tomato plants and were placed in a randomized design. Potted plants in the greenhouse were exposed to temperature conditions following the seasonal pattern of April 2015 in Cairo, Egypt.

Salinity Treatment

Five days old seedlings were transferred to soil minirhizotron and watered with 100 ml tap water every two days for one week. In the second week, the 10 minirhizotron plates were randomly divided into two groups. The first group was the control group and was watered with 100 ml of ¼ MS medium (Murashige and Skoog 1962) every two days for one week. The second group was treated every two days with ¼ MS medium containing 125 mM NaCl also for one week.

Regarding the pot experiment, tomato seeds were planted in 6 pots and left to grow for 12 days and were irrigated with tap water every two days. After that, the pots were randomly divided into two groups. The first group was the control group and watered with approximately 1 L of tap water per pot every two days for one week. Regarding the second group, each pot was irrigated every two days with 1 L tap water containing 125 mM NaCl also for one week. Then, plants were harvested to analyze contents of sodium and potassium, photosynthetic pigments, proline, and for RNA extraction to study the expression of *SOS1*, *NHX3* genes.

Plant Biomass

The fresh and dry weights were determined with the accuracy of 0.001g on the regular lab scale. The dry weight of plant tissues were measured after drying the material for 48 h at 70°C.

Sodium and Potassium Measurements

For the quantification of internal cation contents, the samples were dried in a stove at70 ºC for 48 h then weighed. The internal cations were extracted by adding to the plant samples an extraction solution containing 0.1M HCl and 10 mM $MgCl_2$. The determination of cation content was documented in a flame photometer (GENWAY PFP-7).

Proline and Photosynthetic Pigments Measurements

For the quantification of proline, 0.5 g fresh leaf material from control and salt-treated plants were used. Extraction and quantification were conducted according to the method of Bates *et al.* (1973). The photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids) were determined according to Nornai (1982).

Image Capturing and Analysis of Root System Architecture (RSA) Parameters

Minirhizotron plates of 20 d old tomato seedlings were scanned with a Canon MG2400 series Scanner at 200 dpi. The RSA parameters quantified using EZ-Rhizo software (Armengaud 2009) are total root size, main root length, number of lateral roots, cumulative length of lateral roots, length of branched zone and length of apical zone.

RNA Extraction, Complementary DNA Synthesis and Quantitative Real Time PCR

RNA of three different root samples (50 mg) for control and salinity treatments were extracted using the Ultra Clean Plant RNA Isolation Kit (Jena Bioscience, Thüringen, Germany) according to manufacturer's instructions. Genomic DNA was removed from the RNA using DNase I and then the pure RNA was used to synthesize cDNA using oligo d(T) primers and a SuperScript III First-Strand Synthesis System for RT-PCR kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Gene specific primer sequences for the tomato *SOS1* and *NHX3* genes were synthesized according to Almeida (2014) as shown in table 2. Constitutively expressed 18S rRNA gene was used as positive housekeeping qRT- PCR control for each experiment as described by Nicot *et al.* (2005). The 18S rRNA specific primer sequences were used to yield a 101 bp fragment. Quantitative real time PCR was performed using the Stratagene Mx3000P RT-PCR system as described by the manufacturer (Stratagene, La Jolla, CA) with 85 ng/reaction of cDNA for all genes with the following program: 95°C for 1 min and 45 cycles 95°C for 15 s/ 60°C for 45 min. qRT-PCR was performed in three biological replicates and each reaction repeated twice. DNA accumulation was detected by SYBR Green and the Ct value was calculated using the software provided with the Stratagene Mx3000P RT-PCR system. Dissociation curves showed amplification for only one product for each primer set. Data analysis was performed according to the geometric mean method described by Silver *et al.* (2006) for mRNA expression level detection by delta Ct (ΔCt) values calculation.

The fold change of the *SlSOS1* and *SlNHX3* transcripts level was calculated using specific equations ($\Delta Ct = Ct_{target} - Ct_{housekeeping}$ genes) using the geometric mean of 18S rRNA housekeeping gene for normalization.

Statistical Analysis

The hypothesis of zero difference between means was tested with t test to determine the effect of salinity stress on all the measured and calculated parameters of the experiments. Differences were considered statistically significant when $P<0.05$ in all analyses. Graphs and statistical analysis were carried out using Microsoft Office Excel 2007 software and SPSS statistical software.

RESULTS

Biomass and Relative Water Content

Salt-stressed plants grown in minirhizotrons showed a significant reduction in root fresh weight (FW) after the period of salt treatment, while the reduction of shoot fresh weight was non-significant. Also, shoot/root ratio showed reduction in response to salt stress as shoots is affected by salinity than more roots. (Table 2). Also, as shown in table 3, salinity significantly decreased relative water content in leaves of tomato plants in comparison to control plants.

K^+, Na^+ Contents and Na^+/K^+ Ratio

The results that are shown in tables 4 and 5 display that there was no significant difference between control and salt treatment in regard to the content of K^+ either in leaves or root. On the other hand, salinity significantly increased the content of Na^+ in both shoots and roots; which was reflected as a significant increase in Na^+/K^+ ratio under salinity conditions in both shoots and roots when compared to control treatment. It is worth mentioning that the accumulation of Na^+ in leaves and roots of cv. Agyad-16 under salt stress was almost four and two folds higher in comparison to control, respectively.

Root Growth Rate

Table 6 shows the growth rate of roots of tomato plants under normal and salt conditions. Salinity resulted in a significant decrease of roots growth rate when compared to control condition. The growth rate of root declines to 27 % and 33 % after 14 days and 16 days, respectively.

Root System Architecture

The images of tomato roots (figures 1 and 2) were analyzed using EZ-Rhizo software, and the resulting data of RSA parameters are shown in table 7. Salinity resulted in an inhibition of root growth as shown in many previous studies (West *et al*., 2004). This inhibition was reflected by significant reductions in total root size, main root length, cumulative lateral root length, number of lateral roots, and length of branched and apical zones. Remarkably, the severity of inhibition varied among the measured root parameters. The data clearly showed that, cumulative lateral root length was inhibited more severely when compared to the other root parameters. This was obvious as the decrease in cumulative lateral root length in response to salt stress reached 76 %, followed by the reduction of main root length reaching up to 26.8 %. Also, the decrease in number of lateral roots was very severe, reaching 71 %.

Proline and Chlorophyll Content

Data in table 8 shows that the proline content in tomato leaves increased significantly in plants treated with 125

Mm NaCl when compared to control conditions. On the other hand, salt stress did not change significantly photosynthetic pigments in leaves of tomato plants when compared to control (table 9).

SlSOS1 and *SlNHX3* Genes Expression in Roots

Fold increase of *SlSOS1* and *SlNHX3* genes expression is presented in figure 3. It is clear from the results that salinity resulted in a slight increase in *SlSOS1* and *SlNHX3* expression of 0.3 and 0.6 times, respectively.

DISCUSSIONS

In the present study we investigated the effect of salt stress on RSA in one of the most promising and widely cultivated Egyptian tomato hybrids; Agyad-16. To our knowledge this is the first study to focus on the plasticity of root system of this tomato Egyptian hybrid under salt stress.

Relative water content (RWC) is a measure of water deficit in the leaf that reflects the dynamic water balance between water flow into and out of the tissue (Sinclair and Ludlow, 1985) and, thus, indicating the level of stress that plants are subjected to. It is clear as illustrated in Table 4 that salinity negatively affected RWC of leaves of tomato plants. RWC decreases to 68 % which means that the plants are exposed to high level of salinity during this period and approach severs conditions under normal conditions, RWC values ranged between 90 to 80 %, meaning that soil water is plentiful and the root uptake rate readily matches the transpiration rate. However, a lower RWC suggests that the water deficit produced under salinity stress led to a decrease in water uptake rate by roots in a way that could not match the demands of transpiration, causing a gradual dehydration of cells that may lead to death with prolonged exposure to the stress.

In light of the above, it is clear that the severe salinity level used in our experiments (125 mM NaCl) had major effects on biomass (Table 2). Similar reductions in fresh weight under salinity stress have been investigated by several scientists in tomato (Mozafariyan *et al.*, 2013; Almeida et al. 2014) Afzal *et al*. (2005) on wheat plants. The decline in fresh weight detected due to increased salinity can be attributed to a combination of osmotic and specific toxic ion effects of Cl^- and Na^+ (Basal, 2010), causing disturbances in physiological and biochemical activities which ultimately reduce leaf area and number (Craine 2005; Yunwei *et al*., 2007).

It is also evident from our results that salt stress inhibited root growth, where the total root size (TRS), main root length (MRL), lateral root length (LRL), number of lateral roots and root size were severely affected (Table 7). This was in agreement with previous results which illustrate a significant reduction of MRL, LRL length and TRS in response to high salt stress (Zolla et al. 2010) or deficient plant nutrition conditions (Duan *et al*., 2013). These reductions in root parameters in our experiments might be attributed to the inhibition of cell cycle progression and reduction in root apical meristem size (West *et al.*, 2004), while the decrease in number of lateral roots under salinity may be attributed to ABA signaling (Zolla *et al.*, 2010).

Moreover, tomato plants accumulated significantly higher proline contents in their leaves in response to salt stress (Table 8). During salt stress, plants face both osmotic and ionic stress. To face this problem, the cell adjusts its osmotic potential by accumulating many compatible solutes (i.e., proline, trehalose, and glycine betaine), which not only maintain the osmotic potential of the cell, but also protect the plant by scavenging free radicals, preventing protein degradation, maintaining membrane stability, and mediating signal transduction (Hare *et al*. 1998).

Although the salt stress imposed in our experiments was severe, nevertheless, total chlorophyll contents of tomato leaves were not significantly reduced (Table 9). This result is in accordance with results found by Taffouo *et al.* (2010) where they reported that the supply of mineral nutrient solution with 100 mM NaCl did not affect significantly leaf total chlorophyll content and plant organs dry weight of tomato (cv. Lindo), concluding that the cultivar was relatively more salinity tolerant than other studied cultivars. In addition, it was found in some salt sensitive crops such as rice, that the lower decrease in chlorophyll contents recorded in some cultivars implies that they can grow and perform well under moderately salt stress (Chandramohanan *et al*., 2014). On the other hand, salt stress inhibits the chlorophyll contents in leaves of many crops (Parida and Das 2005).

Salt treatment increased significantly Na^+ contents in roots and leaves of plants. According to Greenway and Munns (1980), NaCl is the predominant form of salt in most saline soils affecting the uptake of many nutrients. Taffouo *et al.* (2010) found that salinity increases Na^+ and Cl^- and decreases K^+, Ca^{2+} and K^+/Na^+ in plant leaves. The high concentration of Na^+ ions interferes with the biological processes required for maintaining the growth of plant cell. For example, it was found that existence of excess sodium cations in cytosol compete with K^+ in binding to enzymes resulting in affecting protein synthesis and activity of key metabolic enzymes (Munns and Tester 2008). In our work, leaves accumulated high concentrations of Na^+ ions under salt condition that reached up to four folds when compared to Na^+ accumulation in control conditions. Nevertheless, some studies show that the link between Na^+ exclusion from the shoots and salinity tolerance is not as clear as previously assumed (Genc *et al.* 2010).

Salt stress has an adverse effect on plant growth. The challenge that faces plants exposed to salt stress is manifested as an osmotic stress and an ionic stress. As a sessile organism, plant is equipped with highly effective mechanisms enabling them to react to improper growth conditions. Plants control Na^+ transport at both tissue and cellular level. This can be achieved by sequestering Na^+ ions in vacuoles where its harmful effect is little. Also, plants can lower the amount of Na^+ that enter the plant through roots (Tester and Davenport 2003; Munns and Tester 2008). Moreover, Na^+ exclusion from the shoots has often been found to be an important mechanism in plant salinity tolerance (Flowers 2004). Genes of *HKT* and *SOS* families play a key role in controlling Na^+ movement throughout the plant. SOS1 transporter, a plasma membrane Na^+/H^+ antiporter, highly contributes in reducing the amount of Na^+ transported to the shoot in transpiration stream and also promote Na^+ efflux (exclusion) from cells back into the external medium (Qiu *et al.* 2002a; Shi *et al.* 2002). In our results we found that the expression of *SOS1* in tomato roots increased in response to 125 mM NaCl treatment. This is in harmony with several previous study on many crops. For example, tomato plants showed a high expression of *SlSOS1* in the roots in response to salt stress (Almeida *et al*., 2014). In general, a higher expression of *SOS1* implies a lower Na^+ concentration in the plant. This was documented to be true in a study of Arabidopsis ecotypes where it was found that ecotypes with high root *AtSOS1* expression under salt-stressed conditions showed significantly lower total plant Na^+ than ecotypes that shows low expression of *AtSOS1* having high total amount of plant Na^+ (Jha *et al.*, 2010). Additionally, the expression of *AtSOS1* in the cells surrounding the vasculature also suggested a role of this transporter in the control of long-distance Na^+ transport in plants, as this ion move from the root to the shoot via the xylem. All in all, the coordination among the different ion transporter determines the amount of salt that will be confining in the roots or ascending up to leaves or sequestered in the vacuole.

Another ion transport protein with a very important role in salinity tolerance is the vacuolar Na^+/H^+ antiporter NHX1. This transporter is responsible for the detoxification of the cytoplasm by sequestring Na^+ into the vacuole (Aharon

et al., 2003) Several studies show that overexpression of *AtNHX1* or its homologs from other species improve the salt tolerance of plants (Chen *et al*.,2008). In this regard, Almeida *et al*., (2014) observed that high expression of *SlNHX1* and *SlNHX3* in roots correlated with low accumulation of Na^+ in the leaves and high accumulation of Na^+ in the roots, respectively. In our findings we found that salinity increase the expression of *NXH3* in roots. In addition, Maathuis *et al*., (2003) studied the expression of *NHX1* and *NHX3* in *Arabidopsis* and found that salt treatment led to up-regulation of transcripts after 10 h, but this relaxed back to control levels after 4 days. Also, expression of *NHX3* was found to be predominantly expressed in root tissues, and did not change during the first 10 hours of salt treatment. However, they found that after 24 the expression of *NHX3* was induced over 10 folds, with some subsequent relaxation of expression level as was observed for *NHX1*. Both strategies of salt stress tolerance are important in tomato, where SOS1 excludes access Na^+ to the medium and at the same time it use tissues tolerance by sequestering Na^+ ions in the vacuole using NHX3. In addition it is notable that the expression level of *NHX* fluctuates up and down coinciding with the amount of Na^+ in cytoplasm and vacuoles. *NHX* up-regulation appears to synchronize with the intermediate period of salt treatment when root vacuoles accumulate Na^+ ions. When Na^+ concentration in the root tissues does not increase any more, down-regulation of *NHX* genes is observed (Maathuis *et al.* 2003).

Table 1: Primers Used in the QRT-PCR Reactions to Measure the Transcript Level of the *SLSOS1* and *SLNHX3* Genes in Tomato Roots. Sequence of Primers are 5´To 3´

Name	Sequence
SlSOS1-F	CCAACCTGCTAAAGTCGTCATTG
SlSOS1-R	CTTGTCTTGTAATCGCGTGTGTG
SlNHX3-F	AGGGGAGGCCATACTCAGTT
SlNHX3-R	TACCCCTGTGCTGAAAAGGA
18S rRNA-F	GGGCATTCGTATTTCATAGTCAGAG
18S rRNA-R	CGGTTCTTGATTAATGAAAACATCCT

Table 2: Fresh Weight (mg) Biomass and Shoot: Root ratio of Agyad-16 Tomato Plants Under Control and 125 mM NaCl Treatment after 20 Days in Minirhizotron

	Shoots	Roots	Shoot: Root Ratio
Control	0.10 ± 0.024	0.03 ± 0.001*	3.85 ± 1.03
125 mM NaCl	0.03 ± 0.003	0.01 ± 0.001	2.79 ± 0.02

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

Table 3: Relative Water Content (%) of Agyad-16 Tomato plants under Control and 125 mM NaCl Treatments after 20 Days in Pots

	Relative Water Content (%)
Control	83.8 ± 0.4*
125 mM NaCl	68.2 ± 0.8

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

Table 4: Contents of K^+, Na^+ (Nmol/mg Dry Weight) and Na^+/K^+ Ratio in Leaves of Agyad-16 Tomato Plants under Control and 125 mM NaCl Treatment after 20 Days in Pots

	K^+	Na^+	Na^+/K^+
Control	399 ± 75	242 ± 49*	0.53 ± 0.03*
125 mM NaCl	370 ± 28	1247 ± 154	2.79 ± 0.03

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

Table 5: Contents of K^+, Na^+ (Nmol/mg dry Weight) and Na^+/K^+ Ratio in Roots ofAgyad-16 Tomato Plants under Control and 125 mM NaCl Treatment after 20 Days in Pots

	K^+	Na^+	Na^+/K^+
Control	441 ± 161	481 ± 79*	0.86 ± 0.14*
125 mM NaCl	271 ± 83	966 ± 207	3.68 ± 0.36

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

Table 6: Main Root Length (cm) of Agyad-16 Tomato Plants under Control and 125 mM NaCl Treatment after 20 Days in Minirhizotron

	4 d	18 d	20 d
Control	2.5 ± 0.7	15.6 ± 1.3*	18.1 ± 1.9*
125 mM NaCl	2.4 ± 0.4	11.3 ± 0.9	12.0 ± 1.2

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

Table 7: Tomato Agyad-16 Root System Architecture Parameters Obtained from EZ-Rhizo and their Response to Salinity Stress after 20 Days in Minirhizotron

Trait Identifier	Trait Description	Control	125 mM NaCl
MRL	Main Root Length (cm)	17.74 ± 2.63	12.98 ± 0.36*
NoLR	Number of Lateral Roots per Main Root (#)	3.67 ± 0.67	1.00 ± 0.00*
Branched	Length of Branched Zone (cm)	6.64 ± 4.57	3.45 ± 3.45
Apical	Length of Apical Zone (cm)	9.13 ± 2.01	5.80 ± 3.05
TRS	Total root size	48.65 ± 4.10	20.31 ± 2.10*
LRL	Cumulative Length of Lateral Roots (cm)	30.92 ± 1.47	7.32 ± 1.73*

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

Table 8: Proline Content (mg/g Dry Weight) in Leaves of Agyad-16 Tomato Plants under Control and 125 mM NaCl Treatments after 20 Days in Pots

	Proline
Control	0.5 ± 0.10*
125 mM NaCl	1.13 ± 0.07

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

Table 9: Photosynthetic Pigments Content (mg/g Fresh Weight) in Leaves of Agyad 16 Tomato Plants under Control and 125 mM NaCl Treatments after 20 Days in Pots

	Total Chl.	Chl. a	Chl. b	Total Carotenoids
Control	0.76 ± 0.07	0.54 ± 0.05	0.22 ± 0.02	0.31 ± 0.03
125 mM NaCl	0.57 ± 0.01	0.40 ± 0.01	0.17 ± 0.01	0.23 ± 0.01

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) are significantly different ($P \leq 0.05$) according to t-test.

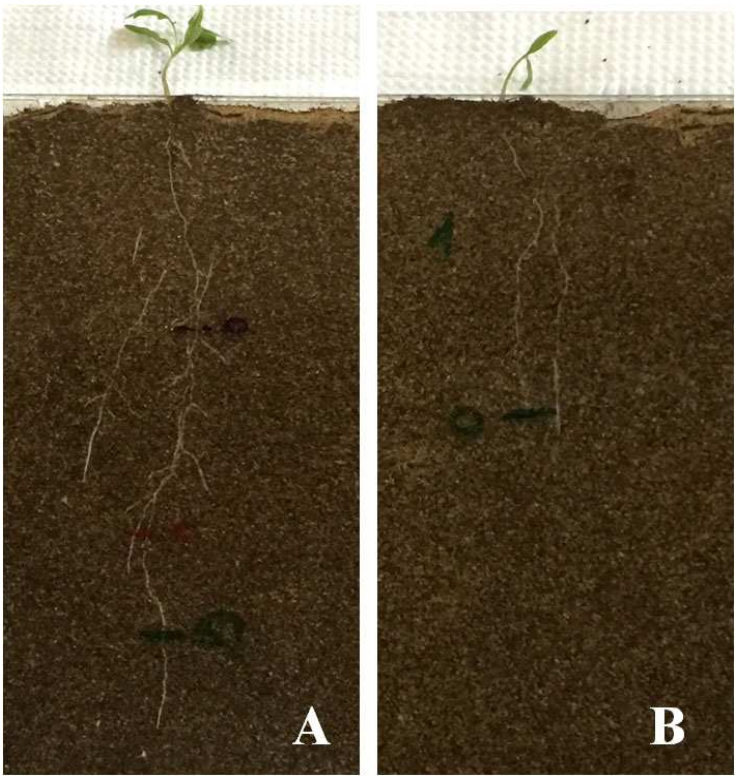

Figure 1: Root Morphology of Agyad-16 Tomato Cultivars under Control (A) and 125 mM NaCl (B) Conditions in Soil Minirhizotron Experiment

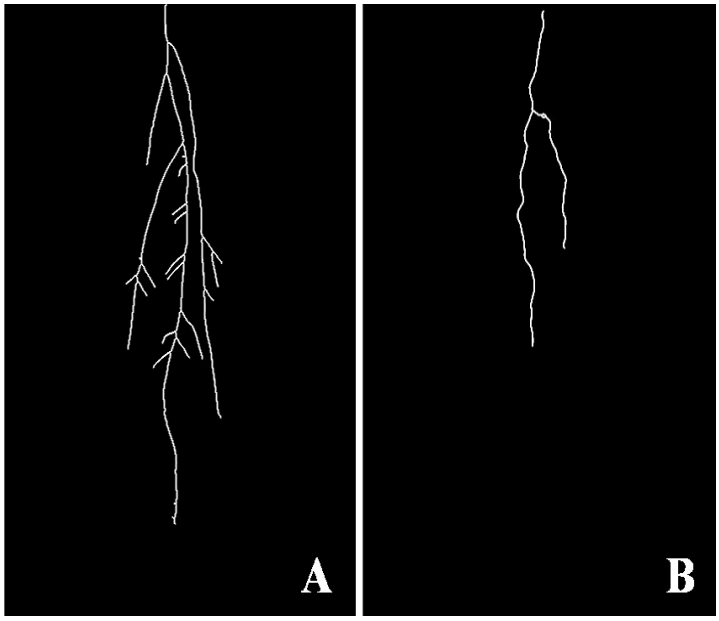

Figure 2: Processed Photos of Root Morphology by Ez-Rhizo Software of Agyad-16 Tomato Plants under Control (A) and 125 mM NaCl (B) Conditions in Soil Minirhizotron Experiment

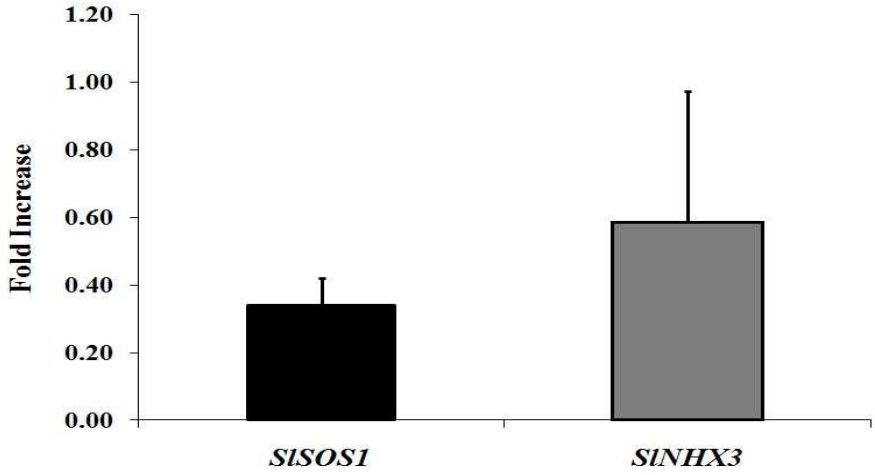

Figure 3: Fold Increase in Gene Expression of *SlSOS1* and *SlNHX3* in Roots of Agyad-16 Tomato Plants under 125 mM NaCl. Gene Expression Results were Obtained using the ΔΔCt Method. Values Indicate the Fold Change ($2^{(-\Delta\Delta Ct)}$) of Genes Expression of an Experiment with three Biological Replicates

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